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14. ABSTRACT
Choroid plexus tumors (CPTs) are intraventricular neoplasms with an undefined molecular profile. Elucidating the genomic landscape of these rare tumors will improve current diagnosis and therapy. 61 fresh frozen CPTs were obtained from a multi-institutional tissue/ clinical database established in our lab. Copy number and gene expression was assessed using Affymetrix GW SNP6.0 and Human Exon 1.0ST arrays, respectively. Methylation was examined using Illumina 450K arrays. DNA from a validation cohort of 20 fresh frozen and FFPE tumors was assessed using OncoScan microarrays. TP53 was sequenced in all samples. CN analysis revealed recurrent choromosomal gains with very few losses in CPPs and aCPPs, while CPCs exhibited many chromosomal gains and losses. Unsupervised clustering distinguished between CPT subgroups using gene expression and methylation intensities. Hypermethylation was observed in CPCs compared to CPPs and aCPPs. Two novel subgroups were identified in CPCs based on genomic content: hypodiploid (n<2) and hyperdiploid (n>2). In hyperdiploid CPCs, > 70% of chromosomes underwent UPD. Hyperdiploid CPCs exhibited worse overall survival; this effect was most significant when the tumor carried a TP53 mutation (3-year OS: 0% vs. 80 p=0.013). The integrative analysis conducted in this study defines, for the first time, the molecular landscape of CPTs and subclassifies CPCs with distinct molecular profiles and clinical outcomes. A greater understanding of the molecular alterations of these tumors will facilitate a more personalized approach to their management.

15. SUBJECT TERMS
Copy number alteration, chromosome 1, copy number driven gene expression, tumor progression

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Key Research Accomplishments from Dr. Malkin toward Award # W81XWH_10-1-0672_CA093469: Molecular Targeted Therapies of Childhood Choroid Plexus Carcinoma (CPC)

INTRODUCTION

Choroid plexus carcinoma (CPC) is a rare malignant brain tumor originating from the epithelial cells lining the cerebral ventricles. CPC represents less than 0.6% of brain tumors in all age groups, yet is more frequent in children (2-4%), especially in infants under the age of 1, accounting for over 20% of brain tumors in this age group (1). T|he molecular events that drive the malignant progression of this tumor are not well understood, yet this knowledge is crucial to improve patient survival. Surgical resection combined with neo-adjuvant and/or adjuvant therapy remain the primary methods of treatment for CPC; however tumor progression and relapse is observed in ~70% of cases (2). Despite improvements on the most current treatment protocols, long-term survival of CPC patients remains under 30% and survivors display significant neurocognitive and/or sensory deficits. (2,3). Identifying altered genes that drive the progression of CPC will refine current diagnostic and prognostic classifications of CPC patients, and promote the implementation of targeted therapies to improve patient survival and reduce long-term side effects. The proposed research aims to identify genetic lesions involved in CPC tumorigenesis in order to implement their use as unique markers for diagnostic and prognostic classification of choroid plexus tumor patients, as well as to promote the creation of personalized molecular targeted therapies.

I hypothesize that recurrent genetic lesions accompanied by a significant change in gene expression in CPC, will be drivers of tumorigenesis in this malignant brain tumor. Consequently, this research project will seek to answer the following question: What genes may be used as molecular markers for diagnostic and prognostic classification of CPC, and for which tumor-promoting alterations could CPC therapies be created?

BODY AND KEY RESEARCH ACCOMPLISHMENTS

We have made considerable progress in the third 12 month period of our project; completing all the elements proposed in our Statement of Work (SOW). We have maintained twice monthly webinars and data sharing exchanges with the group at St. Jude throughout the last funding period. In addition, we had an extremely productive one-day visit (August 2013) on site at St Jude for a complete review and update of the projects. These meetings have proven extremely important, ensuring that we exchange data and ideas in real time, maximizing the efficiency of all project interactions. The following narrative is set in the context of our group SOW, highlighting specifically those tasks assigned to Dr. Malkin. Sections of the original SOW are shown in italics. Details of tasks completed in year 1 follow in Roman text and details of tasks completed in **year 3** are in **bold, underlined text**. A comprehensive manuscript is in preparation that focuses primarily on those tasks assigned to the Malkin group (with other joint manuscripts also in preparation related to the other projects in which the Malkin group played a more collaborative role).

SOW Specific Aim 1, Task 1

Specific Aim 1: To identify candidate drug targets of CPC.

Task 1: Generation of additional human and mouse CPC genomic profiles (timeframe: *months 1-5****).***
The goal of these studies is to expand our number of genomic profiles (DNA and mRNA arrays) of both human and mouse CPCs to provide a comprehensive dataset with which to identify key candidate oncogenes, tumor suppressor genes (TSGs) and aberrant signal pathways that underlie the development of CPC. ***NB. Dr. Malkin has continued to generate human genomic profiles and compare these to mouse CPC profiles throughout the duration of the grant.***

Task 1.d.: *Identify and classify each of the copy number alterations (CNAs) in initial cohorts of human and mouse tumors as 'frequent' (>20% of tumor samples), moderately frequent (>10% to <20%), or rare (<10%) and according to distribution between human TP53 wild-type or mutant tumors and among the different stages of mouse tumor development.* ***PIs:*** *Gilbertson, Malkin, Guy and Ellison.* ***Timeframe:*** *months 4-5.*

Task 1.e: *Accrue and perform central histology review and Affymetrix 6.0 SNP and Exon expression arrays of an additional human CPCs during the course of the granting period.* ***PIs:*** *Malkin, Ellison, Gilbertson.* ***Timeframe:*** ***months 1-36 (ongoing throughout grant period)****.*

Milestone #1: Completion of initial human and mouse CPC genomic datasets*.*

Work completed toward Specific Aim 1, Task 1 by Dr. Malkin

Generation of additional human CPC genomic datasets

- DNA and RNA extraction was conducted for more than 90 human choroid plexus samples using well-established protocols.
- A variety of quality control tests were run to assess the integrity and quality of all samples. Fifty-nine DNA samples were selected for hybridization to Affymetrix SNP 6.0 GW microarrays, while 40 RNA samples were selected for reverse transcription reactions yielding cDNA for hybridization to Affymetrix Exon 1.0 ST microarrays.
- SNP and Exon microarrays were run at the Toronto Centre for Applied Genomics (TCAG) microarray facility.

This represents a significant contribution in the form of additional new human tumor data toward the analysis of CPCs. These data have been used directly to complete Task 2.

SOW Specific Aim 1, Task 2

Task 2: Genome-wide cataloging of candidate oncogenes and TSGs in human and mouse CPCs (Timeframe*: months 5-8).*

The goal of these studies is to identify candidate oncogenes and TSG that map within the CNAs detected in human and mouse CPCs.

Task 2.a: *Catalog all genes located within the frequent, moderately frequent and rare CNAs identified in human and mouse CPCs.* ***PIs:*** *Gilbertson and Malkin.* ***Timeframe****: months 5-6.*

Task 2.b: *Rank probability of the human and mouse genes within all inferred CNAs as being candidate oncogenes or TSGs of CPC.* ***PIs:*** *Gilbertson and Malkin.* ***Timeframe:*** *months 5-6.*

Task 2.c: *Select the top 30 candidate oncogenes and TSGs of human and mouse CPCs.* ***PIs:*** *Gilbertson, Malkin, Ellison and Guy.* ***Timeframe:*** *month 6.*

Task 2.d: *Real-Time PCR and FISH validation of copy number alterations of our lead candidate oncogenes and TSGs in human and mouse CPCs.* ***PI:*** *Ellison.* ***Timeframe:*** *months 6-8.*

Milestone #2: Selection of the top candidate oncogenes and tumor suppressor genes of CPC.

Work completed toward Specific Aim 1, Task 2 by Dr. Malkin

Genomic and transcriptomic analyses to identify candidate tumor suppressor genes and oncogenes

All raw data generated for each DNA and mRNA tumor microarray was used for copy number and gene expression analysis. These are now all complete and cataloged for alterations as detailed below.

Copy Number Analysis-Task 2a.

- Copy number analysis was performed using Partek Genomics Suite (PGS, Partek Inc. St. Louis, MO) and visualized using Integrative Genomic Viewer (IGV, http://www.broad.mit.edu/igv/)
- Normalization of all data was then performed with SNP 6.0 files created for the third phase of the International HapMap Project (HapMap3, 1301 control samples)
- We identified all areas of copy number alteration (CNA) that might contain oncogenes and tumor suppressor genes using the Hidden Markov Model (HMM) and Partek's segmentation algorithm. The minimum number of markers per region was set at 10.
- Candidate CNAs were statistically reviewed by a rigorous set of criteria to prioritize regions of gain or loss for further analysis.

Characterization of allele-specific copy number alterations in choroid plexus tumors

- **Allele-specific copy number alterations were examined using the widely used ASCAT software program (Van Loo et al. 2010, PNAS). Using genotyping array data, the program is able to identify allelic imbalances in addition to copy number changes. This analytical tool allowed us to examine acquired uniparental disomy (aUPD) in CPTs, events characterized by chromosome-wide copy number-neutral loss of heterozygosity.**
- **We found that choroid plexus papillomas (CPPs) and atypical choroid plexus papillomas (aCPPs) exhibited more gains than losses (Table 1). Moreover, we found that frequency of alterations increases with tumor grade (CPP exhibiting the least number of chromosomal alterations followed by aCPPs and CPCs).**
- **One hundred percent of aCPPs (n=8) exhibited gains of chromosomes 7, 11, 12 and 20.**
- **Choroid plexus carcinomas (CPCs) exhibited a large number of gains and losses, as well as a higher number of aUPD, compared to CPPs and aCPPs. The chromosome exhibiting aUPD the most frequently was chromosome 17. Eighty-eight percent of CPCs exhibiting aUPD at chromosome 17 carried *TP53* mutations.**

Table 1: Chromosome-wide alterations in discovery cohort of choroid plexus tumors

Chromosome	Chromosome-wide losses			Chromosome-wide gains			Acquired Uniparental Disomy		
	CPC	CPP	aCPP	CPC	CPP	aCPP	CPC	CPP	aCPP
1	4.3%	0.0%	0.0%	43.5%	13.6%	0.0%	8.7%	0.0%	12.5%
2	21.7%	0.0%	0.0%	30.4%	22.7%	25.0%	8.7%	4.5%	12.5%
3	43.5%	0.0%	0.0%	8.7%	9.1%	12.5%	26.1%	0.0%	0.0%
4	21.7%	0.0%	0.0%	17.4%	18.2%	12.5%	17.4%	0.0%	12.5%
5	34.8%	0.0%	0.0%	17.4%	31.8%	37.5%	26.1%	0.0%	0.0%
6	52.2%	0.0%	12.5%	8.7%	4.5%	0.0%	26.1%	0.0%	12.5%
7	30.4%	0.0%	0.0%	39.1%	68.2%	100.0%	4.3%	0.0%	0.0%
8	34.8%	0.0%	0.0%	30.4%	59.1%	62.5%	13.0%	0.0%	0.0%
9	39.1%	0.0%	0.0%	13.0%	40.9%	75.0%	21.7%	0.0%	0.0%
10	13.0%	18.2%	0.0%	21.7%	0.0%	0.0%	13.0%	4.5%	25.0%
11	34.8%	0.0%	0.0%	13.0%	31.8%	100.0%	26.1%	4.5%	0.0%
12	4.3%	0.0%	0.0%	43.5%	63.6%	100.0%	4.3%	0.0%	0.0%
13	30.4%	0.0%	0.0%	30.4%	18.2%	25.0%	8.7%	4.5%	0.0%
14	8.7%	0.0%	0.0%	34.8%	9.1%	50.0%	8.7%	4.5%	12.5%
15	17.4%	0.0%	0.0%	21.7%	36.4%	50.0%	17.4%	0.0%	0.0%
16	34.8%	0.0%	0.0%	8.7%	9.1%	0.0%	21.7%	4.5%	0.0%
17	30.4%	0.0%	0.0%	13.0%	27.3%	37.5%	34.8%	0.0%	0.0%
18	30.4%	0.0%	0.0%	17.4%	36.4%	37.5%	17.4%	0.0%	0.0%
19	30.4%	0.0%	0.0%	26.1%	27.3%	50.0%	13.0%	0.0%	0.0%
20	13.0%	0.0%	0.0%	34.8%	54.5%	100.0%	8.7%	0.0%	0.0%
21	26.1%	13.6%	12.5%	34.8%	18.2%	25.0%	4.3%	9.1%	12.5%
22	43.5%	0.0%	0.0%	17.4%	13.6%	50.0%	26.1%	4.5%	0.0%
X	8.7%	0.0%	0.0%	4.3%	50.0%	50.0%	0.0%	0.0%	0.0%

- **Two subgroups were identified in CPCs according to the type of chromosome-wide alterations. The first subgroup, termed hypodiploid CPC, exhibited many chromosome-wide losses and very few gains. The average ploidy for this subgroup was 1.45. The second subgroup, termed hyperdiploid CPC, exhibited numerous chromosome-wide gains and aUPD. The average ploidy for this subgroup was 2.76.**

Correlation of molecular subgroups with clinical outcomes uncovers CPC subgroup with worse prognosis.

- **When correlating these newly defined molecularly distinct subgroups with clinical outcomes, we discovered that hyperdiploid CPCs have worse overall survival compared to hypodiploid CPCs.**
- **Moreover, accounting for p53 status further stratified CPCs into clinically distinct subgroups, where hyperdiploid CPCs with *TP53* mutations had the worse outcome (0% vs. 80% *TP53* mutant hypodiploid CPCs & 85% *TP53* wildtype CPC, Log-rank test p<0.0001).**

Accrual and preparation of CPT validation cohort-OncoScan arrays

- **Thirty-one CPT samples were collected to be part of the validation cohort. DNA was isolated from fresh frozen or FFPE tissue and sent for hybridization onto Affymetrix OncoScan 2.0 genotyping microarrays.**

- **Only 20 samples passed quality control (15 CPCs, 3 CPPs, 2 CPPs).**
- **Dr. Ruth Tatevossian from Dr. David Ellison's group at SJCRH supervised the hybridization of samples and aided in the analysis of somatic mutations in CPTs.**

Analysis of oncoscan

- **Copy number alterations: Using Log-R ratios (LRR) and B-allele frequency (BAF) data, we were able to identify allele-specific copy number alterations for all 20 CPTs.**
  - **Chromosome-wide gains and losses, and aUPD were annotated for each subgroup (Table 2).**
  - **Recurrent chromosomal events observed in the discovery cohort were also observed in this validation cohort, including (1) increased chromosome-wide gains in CPPs and aCPPs, (2) numerous chromosome-wide gains and losses in CPCs, and (3) a distinct segregation between hypo- and hyperdiploid CPCs according to number of chromosomes lost vs. gained (hypodiploid average chromosomes gained vs. lost: 0 vs. 12.75, hyperdiploid average chromosomes gained vs. lost: 12 vs. 0.83).**
  - **All aCPPs (n=2) exhibited a gain in chromosomes 7, 12 and 20, which was previously observed in the discovery cohort (n=8). The gain of these three chromosomes may be a distinguishing feature of aCPPs. Further investigation is warranted with a larger tumor cohort.**
  - **As observed previously, aUPD was only observed in CPCs and the most frequently chromosomes exhibiting aUPD was chromosome 17, the loci of *TP53*. Both samples (100%) exhibiting aUPD at chromosome 17 carried mutations in *TP53*.**

Table 2: Chromosome-wide alterations in validation cohort of choroid plexus tumors

Chromosome	Chromosome-wide losses			Chromosome-wide gains			Acquired Uniparental Disomy		
	CPC	CPP	aCPP	CPC	CPP	aCPP	CPC	CPP	aCPP
1	0%	0%	0%	20%	0%	0%	0%	0%	0%
2	7%	0%	0%	27%	33%	50%	0%	0%	0%
3	53%	0%	0%	13%	33%	0%	0%	0%	0%
4	20%	0%	0%	7%	0%	0%	0%	0%	0%
5	33%	0%	0%	27%	67%	0%	0%	0%	0%
6	47%	0%	0%	7%	0%	0%	0%	0%	0%
7	13%	0%	0%	40%	67%	100%	0%	0%	0%
8	40%	0%	0%	40%	67%	50%	0%	0%	0%
9	27%	0%	0%	20%	67%	50%	0%	0%	0%
10	20%	0%	0%	0%	0%	0%	0%	0%	0%
11	53%	0%	0%	20%	0%	50%	0%	0%	0%
12	13%	0%	0%	40%	67%	100%	7%	0%	0%
13	40%	0%	0%	7%	67%	0%	0%	0%	0%
14	13%	0%	0%	13%	0%	50%	7%	0%	0%
15	33%	0%	0%	13%	67%	0%	7%	0%	0%
16	47%	0%	0%	7%	0%	0%	7%	0%	0%
17	47%	0%	0%	33%	100%	0%	13%	0%	0%
18	47%	0%	0%	27%	100%	0%	0%	0%	0%
19	47%	0%	0%	27%	33%	0%	0%	0%	0%
20	0%	0%	0%	40%	67%	100%	0%	0%	0%
21	20%	33%	0%	20%	0%	0%	0%	0%	0%
22	53%	0%	0%	20%	0%	0%	0%	0%	0%
X	47%	0%	0%	13%	67%	0%	0%	0%	0%

- **Somatic mutations: The OncoScan FFPE Express 2.0 genotyping microarray offers SNP coverage of 201 known tumor suppressor and oncogenes, as well as copy number and SNP coverage throughout the genome (median backbone spacing of 1 probe per 9kb). We were able to investigate the presence of somatic mutations in 201 cancer genes for our 20 CPT samples.**
  - **CPCs exhibited the most number of non-synonymous mutations compared to CPPs and aCPPs (average: 2.40 vs. 1.33 vs. 0.50, respectively).**
  - **The most commonly mutated genes in CPTs were *BRAF, KRAS, TP53* and *APC*, with 6, 5, 5 and 4 non-synonymous mutations, respectively.**

- **The most common non-synonymous mutation was found at pG12D in *KRAS* (3 CPCs, 1 CPP), followed by 2 non-synonymous mutations found in *BRAF* at pG469E/R, and pI326T (3 CPCs, each).**

- **Chromothripsis: Visualization of copy number alterations in CPCs revealed two samples with one of the hallmarks of chromothripsis, copy number oscillations between 2 or 3 copy number states along the chromosome *(Korbel & Campbell, 2013 Cell)*. Chromothripsis on chromosome 1p was found to affect a primary and recurrence sample of a *TP53* wildtype CPC patient (Appendix 3, Figure 3). Although, this CN phenomenon is suggestive of chromothripsis, sequencing analysis to identify chromosomal rearrangements, another chromothripsis hallmark, is warranted to validate this finding.**

Gene Expression Analysis-Task 2a and b.

- Gene expression analysis was performed using PGS and Gene Pattern (http://genepattern.broad.mit.edu/).
- Four clustering algorithms (principal component analysis (PCA), unsupervised hierarchical clustering (UHC), non-negative matrix factorization (NMF) and Consensus Clustering (CC)), were used to identify tumor subgroups that segregate independently according to their expression profile. In all analyses, a significant segregation was observed between CPC and choroid plexus papilloma (CPP) samples.
- Microarray intensities were analyzed in PGS, using the benign human choroid plexus papilloma (CPP) samples as an expression baseline reference. This analysis highlights expression changes unique to the malignant choroid plexus carcinoma (CPC) phenotype. Significant changes in expression between CPC and CPP were considered as a fold-change of 2 or more. P-value FDR= 0.05 after ANOVA analysis

Copy number-driven expression-Task 2b and c

- To analyze copy number-driven expression in choroid plexus tumors, we merged the copy number for each segmented region obtained from copy number analysis to the gene expression values.
- The data was filtered according to direction of copy number alteration and expression changes. Only those regions with copy number gain and increased expression, as well as copy number loss and decreased expression were selected.
- Further data filtering took into consideration the frequency of copy number-driven events in the CPC group compared to the CPP group, as the focus was on CPC-unique events.
- An initial list of recurrent CPC-unique regions with significant copy number-driven expression was compiled. These regions were observed in at least 56% of CPC samples (n=10/18). Seventy seven CNAs containing candidate oncogenes were identified. Most amplifications map to chromosome 1, but also include on chromosomes 5, 7, 8, 9 12, 14, 15, 17, 18, 19, 20, 21, X. (**Appendix 1,2**)

Validation of regions of copy number gain with FISH

- **FISH was used to validate the copy number gain observed in the catalog of aberrant genes in human chromosome 1.**
- **Probes mapping to human chromosome region 1p34 and 1q21 were created and utilized in fresh frozen paraffin-embedded human CPC.**
- **A small independent cohort of four CPCs was tested and two samples exhibited recurrent copy number gain of both regions, indicating a gain of the entire chromosome 1. Up to 4 copies of each region were observed.**
- **Further FISH analyses will be performed with a larger independent cohort.**

Splicing analysis

- **Twenty-one genes with concurrent copy number gains and increased gene expression were previously identified in human CPCs and the CPC mouse model. Examining the exon data on these genes revealed differences in splicing and transcription initiation sites in *CDC20* and *CLSPN* between**

CPC and CPP samples. These findings are currently being validated by the nCounter Nanostring system.

Validation of copy number and gene expression alterations in chromosome 1p of CPCs

- **Validation of copy number gains in chromosome 1p were conducted by Dr. David Ellison and his group using FISH (Figure 4). FISH data revealed gain of 1p as well as polyploidy or monosomy in several CPC samples. These findings confirmed the copy number gained observed in genotyping microarrays, and the presence of aneuploidy in CPCS.**
  Validation of increases in gene expression of genes in chromosome 1p is currently underway. We have selected to use the nCounter Nanostring system to validate our data since this method provides absolute expression quantification, rather than relative quantification. CPTs are highly aberrant and may not have an ideal reference gene to use for relative quantification, thus performing an absolute quantification will remove any confounding errors introduced by the selection of a reference gene with variable expression.

All data were discussed in real time with the entire group to ensure that we prioritized and selected the appropriate lesions for further analysis (See the reports of Drs. Ellison and Gilbertson in particular). Regions prioritized as special interest in cross species analyses as likely to contain oncogenes of mouse and human CPCs were then passed forward to Task 3.

Together with Drs. Gilbertson and Ellison we have been working to validate in human CPCs a series of alterations that are also observed in mouse tumors. These CNAs that are common to both species are likely to contain critical genes important in the development of these tumors.

Validating regions with qPCR and FISH-Task 2d.

- Validation of genomic and transcriptomic findings in human CPC by qPCR and FISH are currently in progress. Preliminary qPCR results have confirmed copy number gain in at least four CPC samples for the following genes: STMN1 (1p36), EXO1 (1q43), STIL (1p32), ZYG11A (1p32), RAD54L (1p32), and SLC1A7 (1p32).

Validation of regions of overexpression with Real-time PCR

- Real-time PCR was used as an orthogonal approach to validate the overexpression observed in the catalog of aberrant genes in human CPCs
- Human normal total brain RNA was used as the control sample, and *ACTB1* (Actin beta: 7p22) and *ALAS1* (aminolevulinate, delta-, synthase 1: 3p21) as reference genes.
- The overexpression of the following genes in CPCs have been validated:
  - *RAD54L*
  - *ATP6V0B*
  - *DVL1*
  - *GPATCH3*
  - *USP1*
  - *RPA2*
- Further real-time PCR analyses are currently underway.

Allele specific copy number analysis of CPCs

- The highly aberrant genetic profile of CPCs was dissected using a popular software program (ASCAT, Van Loo 2010, PNAS) that identifies copy number changes using SNP microarrays, taking into consideration tumor ploidy and sample heterogeneity.
- ASCAT confirmed previous observations, in that copy number gain of chromosome 1 was identified in more than 63% of the CPCs analyzed, despite the high frequency of tumor aneuploidy in CPCs.

- A subset of CPCs (43%) exhibited a hyperdiploid genome with 3 or more copies of chromosome 1, while another subset of CPCs (43%) exhibited a hypodiploid genome and most of these samples retain 2 copies of chromosome 1.

Pathway analysis of CPCs

- Gene set enrichment analyses were conducted on the original set of CPC for which exon microarrays were created in order to identify biological functions and pathways for which differentially expressed genes are enriched.
- The publicly available GSEA software program (Broad Institute, Cambridge MA), and a defined list of curated pathway gene sets from various online databases were utilized.
- Dataset permutations were conducted (n=1000), comparing enrichment in CPCs and the benign choroid plexus papillomas.
- The most significant datasets enriched in CPCs were:
    - Mitosis (normalized enrichment score=2.61, FDR $p<0.001$)
    - Spindle organization (normalized enrichment score=2.48, FDR $p<0.001$)
    - Kinetochore (normalized enrichment score=2.36, FDR $p<0.001$)
    - *FOXM1* transcription factor pathway (normalized enrichment score=2.32, FDR $p<0.001$)
    - Chromosome segregation (normalized enrichment score=2.25, FDR $p<0.001$)

- NB. Drs. Gilbertson and Ellison have also confirmed CNAs in these same genes in mouse CPCs. These are extremely exciting data since they identify for the first time candidate oncogenes of CPCs that are present in tumors in two separate species. We are now working with Dr. Guy to determine if these genes are targetable with novel therapies.

SOW Specific Aim 1, Task 3

Task 3: Interspecies analysis of gene candidates and pathway alterations common to human and mouse CPCs (Timeframe*: months 6-8).*

The goal of this analysis is to identify a set of common genes that are dysregulated in both human and mouse CPCs. Genes that are non-redundant for CPC tumorigenesis across species are likely to be critical to the disease process.

Task 3.a: *Compare lists of orthologs of candidate oncogenes and TSGs between mouse and human CPC.* ***PIs:*** *Gilbertson and Malkin.* ***Timeframe:*** *months 6-8.*

Milestone #3: Comprehensive understanding of the molecular similarities between human and mouse CPC.

Work completed toward Specific Aim 1, Task 3 by Dr. Malkin

Comparison between mouse and human data-Task 3a.

- The list generated from regions exhibiting copy number-driven expression in human CPC was used to compare equivalent regions in mouse CPC. The cross-species analysis was analyzed by the Gilbertson group.

REPORTABLE OUTCOMES

ABSTRACTS:

1. Merino D, Shlien A, Pienkowska M, Tabori U, **Malkin D**. *Aberrant copy number driven expression in chromosome 1 is involved in choroid plexus carcinoma progression.* EMBL Genome Workshop. Heidelberg, Germany. October 4-8, 2011.

2. Merino D, Pienkowska M, Shlien A, **Malkin D**. *Aberrant copy number-driven expression in chromosome 1 is involved in choroid plexus carcinoma development.* 2011 SickKids Research Institute Retreat. Toronto, ON. November 17, 2011.

3. Merino D, Pienkowska M, Shlien A, **Malkin D**. *Aberrant copy number-driven expression in chromosome 1 is involved in choroid plexus carcinoma development.* Annual Meeting, American Association for Cancer Research. Chicago, IL. April 1-4, 2012.

4. Merino D, Pienkowska M, Shlien A, Tabori U, Gilbertson R, **Malkin D**. *Mutations in p53 distinguish two molecular subgroups in choroid plexus tumors.* Canadian Student Health Research Forum (CIHR) Winnipeg, MB. June 13, 2012. [platform presentation]

5. Merino D, Pienkowska M, Shlien A, Tabori U, Gilbertson R, **Malkin D**. *Mutations in p53 distinguish two molecular subgroups in choroid plexus tumors.* International Society of Pediatric Neurooncology. Toronto, ON. June 23-27, 2012. [platform presentation]

6. Merino D, Pienkowska M, Shlien A, Tabori U, Gilbertson R, **Malkin D**. *Identification Of Molecularly And Clinically Distinct Subgroups Of Choroid Plexus Carcinomas.* Society for Neurooncology (SNO) Pediatric Meeting. Fort Lauderdale, FL. May, 2013 [platform presentation]

7. Merino D, Pienkowska M, Shlien A, Tabori U, Gilbertson R, **Malkin D**. *Molecular subgroups in choroid plexus tumors: the role of p53 and ploidy in the subclassification of choroid plexus carcinomas.* 6th International Mutant p53 Workshop. Toronto, ON. June 15-17, 2013.

CONCLUSIONS

Our data to date indicates differential patterns of gene amplification and deletion between choroid plexus papilloma and malignant choroid plexus carcinoma. Copy number analysis revealed recurrent choromosomal gains with very few losses in CPPs and aCPPs, while CPCs exhibited many chromosomal gains and losses throughout the genome. The most striking chromosomal targets are throughout chromosome 1, and these appear to correlate highly with mouse orthologues. Unsupervised clustering distinguished between CPT subgroups using gene expression and methylation intensities. Hypermethylation was observed in CPCs compared to CPPs and aCPPs. Two novel molecular subgroups were identified in CPCs according to their genomic content: hypodiploid ($n<2$) and hyperdiploid ($n>2$). In hyperdiploid CPCs, more than 70% of chromosomes had undergone uniparental disomy. When correlated with survival, hyperdiploid CPCs exhibited worse overall survival, and this effect was most significant when the tumor carried a *TP53* mutation (3-year OS: 0% vs. 80 $p=0.013$).

Conclusion:

The integrative analysis conducted in this study defines, for the first time, the molecular landscape of choroid plexus tumors allowing for the sub-classification of CPCs with distinct molecular aberrations and clinical outcomes. A greater understanding of the molecular alterations driving the development of these orphan tumors will facilitate a more personalized approach to the management of CPTs.

APPENDIX 1 (Figure 1)

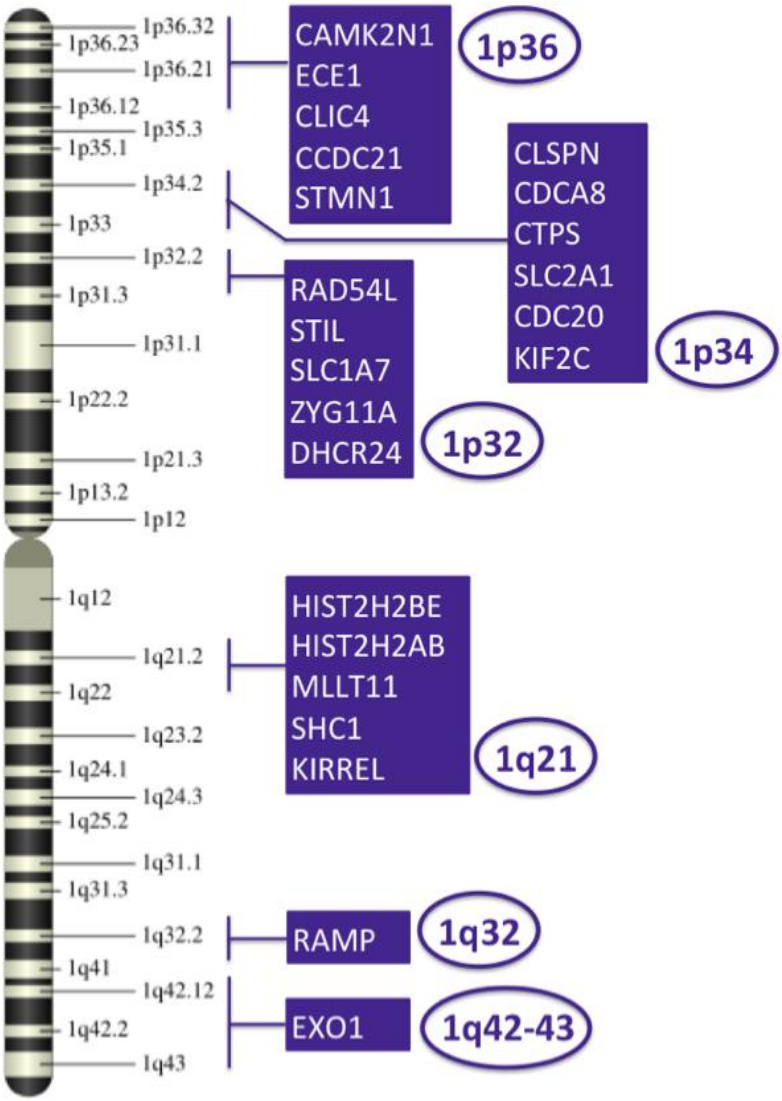

Figure 1: Chromosome 1 contains 23 significant regions of copy number-driven expression unique to CPC.

APPENDIX 2 (Figure 2)

Figure 2: Profile of chromosome-wide CNA in CPP and CPC samples. Black squares represent regions of copy number gain (≥2.75) or loss (≤1.25) that cover more than 50% of each chromosome arm. P-values calculated by Fisher's exact test (significant p-values in red).

APPENDIX 3 (Figure 3)

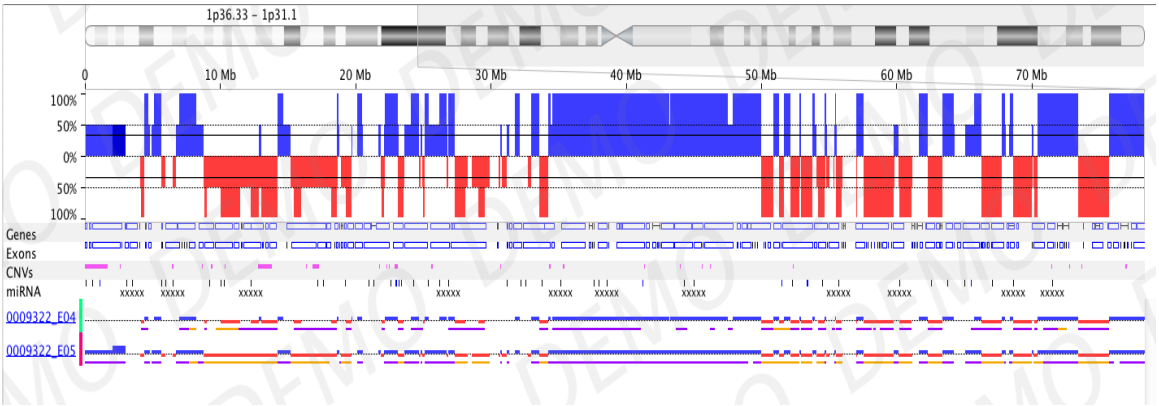

Figure 3: Copy number oscillations (gains in blue, losses in red) in chromosome 1p

APPENDIX 4 (Figure 4)

CPC FISH data

Tumor ID	CNAs
2776	61% T:T:C
2798	polysomy 3-4
3388	polysomy 3-4
2837	polysomy 3-4
4228	polysomy 3-4
0926	polysomy 3-5
4199	no CNA

RAD54L

EXO1

1p control

2776

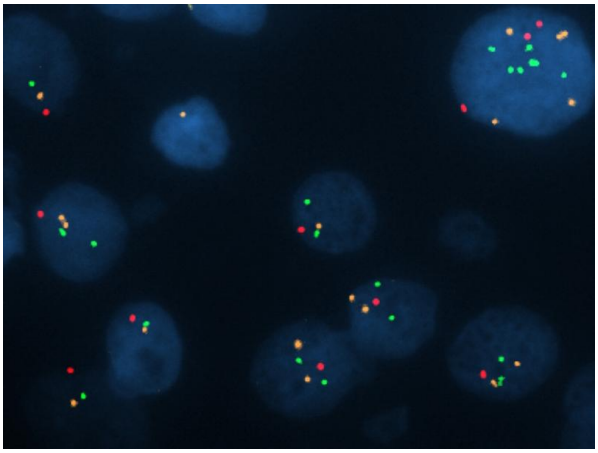

Figure 4: Results for CPC FISH data